C. Remarks

Claims 36-51 are pending. Claims 36, 42 and 46 have been amended. New claims 52 and 53 have been added. Consideration of the amended and new claims is respectfully requested.

The Examiner has rejected claims 36-51 under 35 U.S.C. § 112, second paragraph, as failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Specifically, the Examiner alleges that whereas the claims are directed to a method of purifying a protein, the claims only recite steps for loading and washing a column and lack an endpoint of purification, making the claims appear incomplete.

In response, Applicants have amended the claims to indicate that the protein is purified, making the claims "complete." Support for this amendment is found throughout the originally filed application, and specifically at page 2, lines 12-14 and page 3, lines 15-16. Therefore, Applicants respectfully request withdrawal of the rejection of claims 36-51 under 35 U.S.C. § 112, second paragraph.

Applicants have further amended claims 36, 42, and 46 to better define the subject matter which Applicants regard as the invention. Specifically, the claims now recite that the protein is captured on a chromatography substrate. Support for these amendments and new claims 52 and 53 is found in the originally filed application, for example, at page 1, lines 29-30; page 2, lines 10-11; page 3, lines 13-14; and page 6, lines 14-18, as well as in the originally filed claims. The amended claims encompass both column and batch purification methodologies. New claims 52 and 53 have been added to specifically recite column and batch methodologies, respectively.

The Examiner has rejected claims 46, 47, 50 and 51 under 35 U.S.C. § 102(b) as allegedly anticipated by Burton et al. The Examiner alleges that Burton et al.

teach the purification of anionic proteins (neurotrophins) using hydrophobic interaction chromatography (HIC). Applicants respectfully traverse the rejection.

Burton et al. discloses a method of separating misprocessed, misfolded, size, glycosylated, or charged variants from mature neurotrophins (NGF) using HIC, and thereby purifying the mature NGF. The method of purifying NGF by HIC is specifically described in Examples IV-VI. First, improperly folded NGF from inclusion bodies is obtained and refolded. The refolding procedure includes the step of adding PEI (poly-ethylene-imine) to the sample, which precipitates nucleic acid and other acidic-charged molecules (such as anionic proteins and DNA/histone complexes), and removing the precipitates (i.e., nucleic acids and other acid-charged molecules) by centrifugation. *See* Burton et al., column 30, lines 17-21. Accordingly, the column load in Burton et al. does not contain nucleic acids and other acidic-charged molecules.

In stark contrast, rejected claims 46, 47, 50, and 51 are directed to a method of purifying a protein in a sample from a plurality of DNA/histone complexes, wherein a sample is captured onto a hydrophobic interaction chromatography substrate and DNA is removed during a wash step. Because Burton et al. start with a column load previously treated with PEI to remove such nucleic acids and other acidic-charged molecules (i.e., DNA/histone complexes and anionic proteins), they do not teach a method of purifying a protein from DNA/histone complexes using HIC, wherein the wash step removes DNA. Moreover, for the same reasons, Burton et al. do not inherently teach or anticipate the presently claimed invention.

In fact, Burton et al. teaches away from the present invention. A prior art reference must be considered in its entirety, including portions that teach away from the claimed invention. *See* M.P.E.P 2141.02; *see also W.L. Gore & Assoc., Inc. V. Garlock, Inc.*, 721 F.2d 1540, 220 U.S.P.Q. 303 (Fed. Cir. 1983), *cert. denied,* 469 U.S. 851 (1984). A reference is said to teach away if a skilled artisan looking to the reference would have

taken a different path than Applicant. *See Tec Air, Inc. V. Denso Manufacturing Michigan Inc.*, 192 F.3d 1353, 1360, 52 U.S.P.Q.2d 1294 (Fed. Cir. 1999).

Burton's plain teaching is that nucleic acids and other negatively charged molecules are removed using PEI, thus requiring an additional step for the removal of DNA prior to the HIC step. In contrast, the present invention teaches that DNA may be removed during the HIC step. Skilled artisans looking to Burton et al. would have taken a different path, i.e., they would have included a separate step for the removal of DNA. Therefore, Burton et al. does not anticipate the claimed invention and actually teaches away from the invention.

Burton et al. further disclose that "neurotrophins belong to a family of small, basic proteins" (column 8, line 10; emphasis added). It is well known in the art that basic proteins are positively charged and cationic. This is further evidenced by the fact that Burton et al. teach purifying the neurotrophins by cationic exchange chromatography. Cationic exchange resins bind to positively charged cationic proteins, not to anionic proteins. The proteins of the present invention, however, can be purified using anionic exchange chromatography (*see* page 6, lines 32-38) because they are anionic (e.g., negatively charged) (*see* page 1, lines 15-17; page 4, lines 4-5). Therefore, Burton et al. does not anticipate claim 47. Based on the foregoing remarks, Applicants respectfully request withdrawal of the rejection of claims 46, 47, 50, and 51 under 35 U.S.C. § 102(b). In addition, Applicants note that the Burton et al. patent issued on July 23, 2002, so it is not available as 102(b) art against the present application.

Entry of this amendment is requested since it responds to the rule 112 and 102 rejections raised by the Examiner and obviates them. The amendment therefore reduces the issues and places the case in better form for possible appeal. In view of the foregoing comments and amendments, favorable consideration and allowance of all pending claims is earnestly solicited.

In addition, Applicants note that the Examiner crossed out the entry for the Lottspeich reference (*see* paper returned to Applicants; date considered: March 12, 2003). Applicants understand this marking to indicate that the Examiner did not consider the Lottspeich reference at the time; however, Applicants believe the Examiner should have considered the reference in light of the filing of the International Search Report [see MPEP 609 (III. A(3))], as the Search Report provided a concise explanation of relevance of the document. Applicants herewith provide an English translation of the Lottspeich reference as a convenience for the Examiner. As the Lottspeich reference does not address the subject matter of the currently pending claims, Applicants do not believe the Lottspeich reference will impact the Examiner's assessment of the currently filed amendment.

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Lottspeich, F. (ed.) "Bioanalytik" (1998) Spektrum Akademischer Verlag, Heidelberg, Berlin pp. 208-209

[TRANSLATION]

[Translator's note: End portion of section 9.6 on p. 208, and beginning portion of section 9.8 on p. 209, omitted.]

(p. 208) --- Part I: Protein Analysis

9.7 Hydrophobic Interaction Chromatography

Hydrophobic interaction chromatography (HIC, engl. *hydrophobic interaction chromatography*), is characterized by adsorption of the non-polar surface regions of a protein to the weakly hydrophobic ligands of a stationary phase at high salt concentrations. In contrast to RPC, the elution of the sample molecules is achieved by decreasing the salt concentration. HIC combines the properties of a non-denaturing salt precipitation (aggregation due to hydrophobic protein-protein contact) with the separation ability of chromatography (interaction due to hydrophobic protein-matrix contact). This yields the basic advantage of maintaining the native form and biological activity of a protein.

Precipitation of Proteins Section 2.4

A comparison with salt precipitation is valuable for a closer look at hydrophobic interaction. Increasing the salt content of a solution causes an increase in surface tension, which removes the water shell of the protein, thereby exposing the hydrophobic regions. Proteins in an aqueous environment that has increasing salt concentrations interact via hydrophobic surface areas: More and more molecules "stick" to each other, and are eventually precipitated. The more hydrophobic the proteins are, the less salt is necessary for precipitation or for binding to a hydrophobic matrix. It is striking that in HIC, the separation effect occurs mainly during the adsorption rather than the desorption process, the latter being more typical. It is therefore especially important to optimize the initial conditions, and specify the HIC matrix, the buffer, the pH value, and the salt concentration.

Lottspeich, F. (ed.) "Bioanalytik" (1998) Spektrum Akademischer Verlag, Heidelberg, Berlin pp. 208-209

[TRANSLATION]

Both synthetic polymers and biopolymers, with surfaces that are modified by ligands of varying hydrophobicity, are used as stationary phases in HIC. Typically, alkyl or phenyl groups are used. The selectivity and the capacity of the HIC matrix are thereby essentially determined by the hydrophobicity and density of the ligands. Phenyl groups are also responsive to aromatic interactions, in addition to hydrophobic ones, and thus differ from the chromatographic effect of the alkyl chains. However, the choice of ligands for a separation must be made empirically. The salt used is chosen based on the Hofmeister series, which ranks anions and cations in terms of their support for hydrophobic interactions:

Hofmeister Series Section 2.4

"Salting-out effect"

$\longleftarrow$

Precipitation

$$SO_4^{2-} > CH_3COO^- > Cl^- > Br^- > NO_3^- > ClO_4^- > I^- > SCN^-$$

$$NH_4^+ > K^+ > Na^+ > Cs^+ > Li^+ > Mg^{2+} > Ca^{2+} > Ba^{2+}$$

$\longrightarrow$

chaotropic effect

Certain ions initiate hydrophobic interactions and have a precipatory effect, e.g. ammonium cations. Others prevent interactions, e.g. via the dissociative effect of iodine and thiocyanate anions, and are called **chaotropic**. For HIC, 1 to 2.5 M ammonium sulfate is usually used, whereby the concentration depends on the hydrophobic properties of the individual protein.

The elution occurs with a linear descending salt gradient, or with a step-wise decrease in salt concentration. Additives can be also be included to decrease the protein-ligand interaction. Examples of this are organic solvents (methanol, ethylene glycol) or detergents (e.g. sodium dodecyl sulfate, SDS). The potential influence of the pH value on an HIC separation must be determined for each individual experiment. In general, the hydrophobic interaction is the strongest in the neutral area (pH to 5 to 8.5), since most proteins are uncharged in this range. A decrease in temperature weakens the hydrophobic interaction.